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# A Floral Diet Increases the Longevity of the Coccinellid *Adalia bipunctata* but Does Not Allow Molting or Reproduction

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*Adalia bipunctata* L. (Coleoptera: Coccinellidae) is a generalist aphidophagous coccinellid and an important natural enemy in many agroecosystems including orchards. Coccinellid species have been observed to consume non-prey food like nectar and pollen, but the value of these foods for *A. bipunctata* is poorly known. The objective of this study was to determine the effect of different prey and non-prey diets on *A. bipunctata* larval development and adult longevity and fecundity. Larval development was studied on three prey diets: The aphids *Dysaphis plantaginea* and *Myzus persicae* and Lepidopteran eggs of *Ephestia kuehniella*; five flower diets: *Matricaria chamomilla*, *Daucus carota*, *Fagopyrum esculentum*, *Anethum graveolens*, and *Sinapis alba*; four pollen diets from three plant species: *Typha angustifolia*, *Malus pumila* (two varieties) and *A. graveolens*; and 1 M solutions of three sugars: glucose, fructose, and sucrose. Adult longevity and fecundity were tested on one prey diet (*E. kuehniella* eggs), three flower diets (*F. esculentum*, *A. graveolens*, and *S. alba*); the same four pollen diets and three sugar diets with larvae; and finally a mixed diet of sucrose and *A. graveolens* pollen. A water-only (starvation) control was used for both larval development and adult longevity and fecundity. Adult lipid content was assessed as a measure of how non-prey food affects the ladybeetles' nutritional status. Larvae did not develop beyond the first instar on any of the non-prey diets, but they lived more than twice as long as on *F. esculentum* and sugar diets than on water. Sugar and flower diets improved *A. bipunctata* adult longevity (71–92 days and 10–66 days, respectively) over a pure pollen diet (6–7 days). Fecundity was nil on all non-prey diets, and within a normal range on *E. kuehniella* eggs. The results suggest that pure floral diets do not support *A. bipunctata* molting or reproduction, but flowering plants can prolong *A. bipunctata* larval survival and adults longevity considerably when prey are absent. Adults on sugar diets had high lipid content, indicating that sugar feeding can improve overwintering survival. The findings could be used in agroecosystem design, such as the composition of flower strips for optimal functional diversity.

**Keywords:** non-prey food, *Adalia bipunctata*, conservation biological control, functional biodiversity, lipid content

## INTRODUCTION

The two-spotted ladybird *Adalia bipunctata* L. (Coleoptera, Coccinellidae) is native to Europe, Central Asia and North America (Majerus, 1994), and one of the most common coccinellids in orchards (Doubt et al., 1998; Mehrnejad et al., 2011), preferring arboreal habitats, but also found on herbaceous plants in nature (Leather et al., 1999). It is a commercially available species, widely used for aphid control in many countries (Majerus, 1994; Jalali et al., 2010) and for psyllid control (Khan et al., 2016).

*Adalia bipunctata* is a polyphagous predator with a wide range of prey (Omkar, 2005). *Adalia bipunctata* was observed visiting flowering plants (Free et al., 1975), and pollinivory by *A. bipunctata* in early spring has been detected by gut dissection, especially within the pollen of Rosaceae (Hemptinne and Desprets, 1986). Feeding experiments indicate that pollen represents an alternative food source, enabling females to promptly oviposit at the time of aphid population increase (Hemptinne and Desprets, 1986). Furthermore, Hemptinne and Desprets (1986) reported that *A. bipunctata* larvae completed the development on pollen of Rosaceae alone. In the laboratory, *Harmonia axyridis* (Pallas) can complete development and reproduce on bee pollen alone (Berkvens et al., 2008), whereas *Coleomegilla maculata* (DeGeer) can develop and reproduce on a diet consisting solely of maize pollen (Lundgren and Wiedenmann, 2004). Ladybeetles oviposit in aphid patches (Dixon, 1959; Mills, 1979), but the number of aphids in each patch changes over time, often dramatically, even in the absence of natural enemies (Dixon, 1985). Polyphagy may have served as an evolutionary stepping stone for primarily predaceous groups to adopt new feeding habits (Giorgi et al., 2009), and non-prey foods are probably used by coccinellids to increase survival when prey is scarce (Lundgren, 2009). Thus, the exploitation of non-prey food may expand biological control services by coccinellids.

Plants are sources of pollen and nectar and can provide a habitat for alternative prey and natural enemies too. Flowering plants have been widely used in conservation biological control (Fiedler et al., 2008; Haaland et al., 2011), and can increase natural enemies' longevity, fecundity, and predation or parasitism rates, which in turn can enhance the effectiveness of natural enemies as biocontrol agents (Lee and Heimpel, 2008; Russell, 2015; van Rijn and Wäckers, 2016). The role of pollen and nectar is well studied for hymenopteran parasitoids (e.g., Winkler et al., 2009; Russell, 2015), but increasing attention is being given to the role of non-prey food for predator fitness components such as survival or reproduction, involving studies on coccinellids (Bertolaccini et al., 2008), neuropterans (Resende et al., 2017), predatory mites (Khodayari et al., 2013; Khanamani et al., 2016; Riahi et al., 2017), spiders (Pollard et al., 1995; Nyffeler et al., 2016), and syrphids (van Rijn and Wäckers, 2016). Sugar feeding can improve fitness and performance, as well as nutritional status in coccinellids (Lundgren and Seagraves, 2011; Seagraves et al., 2011).

To contribute to the design of agricultural systems to support *A. bipunctata*, it is important to determine the value of different floral diets and their main sugar constituents for larvae and adults. Five annual flowering plants often used in flower strip

mixtures, which represented four different plant families, were tested. Since *A. bipunctata* is an important predator in apple orchard and has a preference for pollen of Rosaceae (Hemptinne and Desprets, 1986), two varieties of apple pollen were included in the study, as well as cattail pollen (*Typha angustifolia*) and *A. graveolens* pollen. The major components of plant nectars are sucrose, fructose and glucose, occurring in different proportions in different plant species (Baker and Baker, 1983), and their value to a given insect species may differ. Therefore, these three sugars were also included in the study. Prey species used were the rosy apple aphid (*Dysaphis plantaginea* L.), a principal apple orchard pest of which *A. bipunctata* is a known major enemy (Wyss et al., 1999), and the peach aphid (*Myzus persicae* L.), occurring in many crops including orchards. Finally, *Ephestia kuhniella* Zeller eggs were tested, as this is a high-value prey often used in mass-rearing. The objective of this study was to determine the effect of whole floral diets, as well as selected pollens and main sugars, on life history parameters related to *A. bipunctata* immature development and adults' survival, reproduction and overwintering. Lipid content was analyzed since it is important for survival and would serve as a measure of how non-prey food affects the ladybeetles' nutritional status.

## MATERIALS AND METHODS

### *Adalia bipunctata* Rearing

Insects were taken from a laboratory stock colony at the University of Copenhagen, which started in March 2017 with eggs purchased from EWH BioProduction ApS (Tappernøje, Denmark). In the stock colony, *A. bipunctata* larvae and adults were fed on *D. plantaginea* [leaves of *Plantago lanceolata* L. (Lamiales; Plantaginaceae) infested with *D. plantaginea* were offered]. The colony was maintained in plastic containers, with ventilation holes in the lid screened with fine nylon mesh. A soaked cotton plug fitted into an Eppendorf tube served as a source of water. The stock colony was maintained in a growth chamber at  $23 \pm 1^\circ\text{C}$  and a 16:8 h (L: D) photoperiod. For experiments on juvenile development, *A. bipunctata* eggs were collected from the colony and placed in 14-cm Petri dishes in the same growth chamber until hatched, and larvae were isolated after hatching. Newly hatched larvae (<24 h) were used to assess larval development. Larvae were collected from 14-cm Petri dishes and given *E. kuhniella* eggs to feed on. The newly-emerged adults (<24 h) were used for the longevity and fecundity experiment.

### Aphid Rearing

*Dysaphis plantaginea* was reared on its summer host, *P. lanceolata* (Plantain), at  $20^\circ\text{C}$  and 16:8 L: D photoperiod. Aphids originated from collections made on Zealand in 2015–2016 in the University of Copenhagen's Pometum and an organic orchard near Køge (Ventegodtgaard, Lille Skensved). The *M. persicae* culture was maintained on pepper plant *Capsicum annuum* var. *groszum* L. (Solanales; Solanaceae). Both *P. lanceolata* and *C. annuum* were grown in the greenhouse under L: D 16:8 conditions at a minimum of  $20^\circ\text{C}$ .

**TABLE 1** | Information about nectar sugar content and pollen of the five plant species tested.

Species	Sugars, mM/10 mg dry weight of flower						Pollen (protein %)	Corolla		References
	Total sugar	Fructose	Glucose	Sucrose	s/(g+f) ratio	g/f ratio		Depth mm	Width mm	
<i>Anethum graveolens</i> L.	5.5	1.4	1.7	0.5	–	–	Yes	0	–	Irvin et al., 2007
<i>Fagopyrum esculentum</i> L.	2.5	1.3	0.9	0.2	1.5	1.0	Yes (11.4)	0.5	1.0	Somerville and Nicol, 2006; Vattala et al., 2006; Irvin et al., 2007; van Rijn and Wäckers, 2016;
<i>Sinapis alba</i> L.	–	–	–	–	0.02	1.2	Yes	4.5	4.3	Vattala et al., 2006
<i>Daucus carota</i> L.	27.2*	–	–	–	–	1.0	Yes	0	–	Hicks et al., 2016; Broussard et al., 2017
<i>Matricaria chamomilla</i> L.	–	–	–	–	–	–	Yes	–	–	Lunau and Wacht, 1994; Abd et al., 2007

\*( $\mu\text{g/day}$ ) per single flower; – data not available.

## Non-prey Food Plants

Five plant species were selected from four different plant families: Polygonaceae (*Fagopyrum esculentum* L.), Cruciferae (*Sinapis alba* L.), Apiaceae (*Daucus carota* L. and *Anethum graveolens* L.), and Asteraceae (*Matricaria chamomilla* L.) (Table 1). These plants are often found in flower strip mixtures. Plants were grown from seed in 13 cm pots in a greenhouse (L: D = 16:8 h, min. 20°C), and were used when flowering. Cohorts of plants were sown once a week from March to July to ensure a steady supply of flowering plants for the duration of the experiment. *D. carota*, *A. graveolens*, and *M. chamomilla* started flowering in May; while *F. esculentum* and *S. alba* started flowering in June.

## Pollen

Four types of pollen were tested. Two varieties of apple pollen (*Malus pumila* L. Rome and *Malus pumila* L. Red Delicious; Firman Pollen Company, WA, United States); cattail pollen (*Typha angustifolia* L.; Biobest NV, Westerlo, Westerlo, Belgium) and *A. graveolens* pollen collected from flowers grown in the greenhouse. Apple pollen is easy to find on apple leaves in orchard during the flowering period (Addison et al., 2000). Cattail pollen has a high value for predatory mites mainly consisting of the family Phytoseiidae (Samaras et al., 2015), and is currently used in greenhouses (Pijnakker et al., 2015). *A. graveolens* produce abundant pollen, which is easily accessible to insects (Irvin et al., 2007). Pollen was sieved with fine mesh (thread diameter = 39 microns) prior to testing, ensuring that only pure pollen powder was used for the experiment.

## Sugar Solutions

Sucrose, fructose, and glucose are major components of plant nectars (Baker and Baker, 1983), and their nutritional value to a given insect species varies. Not all mono- and oligo-saccharides are equally suitable for coccinellids (Nijijima et al., 1997) therefore, 1 M solutions of two monosaccharides D-(+)-fructose and D-(+)-glucose (Merck KGaA, Darmstadt,

Germany) and a disaccharide D-(+)-sucrose (Nordic Sugar, Copenhagen, Denmark) were also tested.

## Experimental Design

### *A. bipunctata* Larval Development

Fifteen different diets were tested: flowers of five species (*F. esculentum*, *S. alba*, *D. carota*, *A. graveolens*, and *M. chamomilla*), four types of pollen (*M. pumila* Rome, *M. pumila* Red Delicious, *T. angustifolia*, and *A. graveolens*), three sugar solutions (1 M glucose, fructose, and sucrose solutions), two species of aphids (*D. plantaginea* and *M. persicae*), and *E. kuehniella* eggs. For each treatment, 17–46 larvae were tested. Water was provided in all treatments and water-only was the starvation treatment. Larvae (<24 h) were selected randomly and placed in individual 30 ml plastic cups with a piece of fine mesh netting held in place by the rim of a lid with a hole in the center, allowing ventilation. A 1 cm layer of agar (15 g/l) in the bottom of the plastic cup provided moisture. Water and sugar solutions were provided by filling a 0.5 ml microcentrifuge tube, sealed with soaked cotton serving as a dispenser. For the aphid treatments, each cup contained a piece of a leaf from *P. lanceolata* infested with *D. plantaginea* or from *C. annuum* infested with *M. persicae*. Pollen diets were placed on a piece of filter paper on the agar layer, and *E. kuehniella* eggs and flower diets were placed on the agar layer directly. The agar served to support flowers and provided a water source, keeping the flowers fresh. Flowers were collected between 10.00 and 12.00 h while blooming in the greenhouse. Only flowers without any insect infestation were chosen. All food types were provided *ad libitum*. Flowers, pollen, aphids and *E. kuehniella* eggs were replaced daily while sugar diets were replaced every other day and water added if necessary. The plastic cups with the agar layer were replaced with new ones when the diets were replaced.

The developmental stage (first, second, third and fourth instar, pupa, and adult) and the survival of *A. bipunctata* larvae were monitored on a daily basis. The day of molting could be determined by observing the cast skins.

### *A. bipunctata* Adult Longevity and Fecundity

Adults (<24 h) were tested with one of the following diets: Three different flowers (*A. graveolens*, *F. esculentum* and *S. alba*; carrot and chamomile flowers were excluded due to a shortage of flowers), four types of pollen (as above), three types of sugar solutions (1 M glucose, fructose and sucrose solution), *A. graveolens* pedicels (flower removed), and a mixed diet of sucrose solution plus *A. graveolens* pollen. The prey diet of *E. kuehniella* eggs was used as a positive control. Water was provided in all treatments and water-only was the starvation treatment. Rahaman and Aniszewski (2014) reported that *A. bipunctata* can consume young leaves or buds of legume plants when aphids are not available. The pedicel was a plant part available in the flower diet treatments, so *A. graveolens* pedicels were included to test the possible value of the pedicel for *A. bipunctata*, and to serve as an additional negative control. One or two males and one or two females (all <24 h old) were placed together in one container (plastic cup, 6 cm in diameter 7.5 cm height; with a piece of fine mesh netting held in place by the rim of a lid with a hole in the center, as above). A few days after they emerged, adults started to mate. After mating was confirmed by observing first eggs, adults were kept individually in a new 30 ml plastic cup. For each treatment, 25–55 adults were tested, fewer adults (25) were used in the *F. esculentum* flower diet because of a shortage of flowers.

In each container, flowers were provided in a small cylindrical plastic vial with water, plugged with cotton wool to prevent accidental drowning of adults. Flowers were collected between 10.00 and 12.00 h in the greenhouse. Only those without any insect infestation were chosen. Pollen and *E. kuehniella* eggs were placed on the bottom of the container, and sugar solution and water were provided by filling a 0.5 ml microcentrifuge tube, sealed with soaked cotton serving as a dispenser. All food types were provided *ad libitum*. Flowers were replaced daily, *E. kuehniella* eggs, pollen and sugar solution diets were changed every 2–3 days to maintain good quality and avoid the growth of fungi, and water was added if necessary. Containers were replaced with new ones every 2 or 3 weeks.

The survival and fecundity of adults were checked daily; Dead individuals were removed and placed in a  $-20^{\circ}\text{C}$  freezer for lipid analysis. *Adalia bipunctata* eggs were counted and females moved to new cups. Longevity in the *E. kuehniella* treatment was very long, and after 241 days the remaining adults in that treatment ( $n = 20$ ) were freeze-killed. Lipid content of both dead and freeze-killed adults was determined.

### Lipid Analysis

Lipid extraction of *A. bipunctata* adults, who fed on different diets, and newly emerged adults, who fed on *E. kuehniella* eggs at the larval stages, was done in a Soxhlet extractor with petroleum ether (PE) (Williams et al., 2011). Extraction time was 72 h. Prior to extraction, *A. bipunctata* adults were oven-dried at  $50^{\circ}\text{C}$  for 24 h and weighed. After lipid extraction, they were re-weighed to obtain the fat-extracted dry weight. Weighing was done on an XPR Micro and Ultra-Microbalance [readability down to 1 microgram, Mettler-Toledo (HK) MTCN, Hong Kong, China]. The mass of total

lipids was calculated as the weight of each individual sample before extraction, minus the weight of the same sample after extraction.

### Statistics

The Kaplan-Meier (KM) method was used to fit survival curves of each treatment, using the “surv” and “survfit” function from the “survival” package (Therneau, 2014) in R (R Core Team, 2014) and mean survival time and standard error were extracted from the curves using the “print.survfit” function from the same package. The effect of different diets on the longevity of larvae and adults was tested by Cox proportional hazards model (R function “coxph”). For adults, sex was included in models as well as the interaction effect of sex and diet, with cage as a random effect (using function “cluster”) (R Core Team, 2014). Models were reduced by removing higher order non-significant interactions. The “lsmean” function was used to perform pairwise comparisons for each pair of treatments. A General Linear Model (GLM) was used for lipid comparison among treatments and sex, followed by “lsmean” for pairwise comparisons for each pair of treatments. Data are presented as mean values  $\pm$  standard error (SE).

## RESULTS

### Larval Development

Larvae did not develop beyond the first instar in any of the non-prey diet treatments, but the survival of larval was significantly affected by diet (Cox PH,  $df = 15$ ,  $\chi^2 = 287.1$ ,  $p < 0.0001$ ). Larvae lived longer on *F. esculentum* and sugar diets than on other flower and pollen diets (Table 2). None of the four pollen diets differed significantly from the starvation treatment (water-only) and were all significantly poorer than all other diets with a significantly shorter survival time. Flowers of *F. esculentum*, *S. alba*, and *A. graveolens* increased the survival of larvae, especially *F. esculentum*, being slightly better than sugar diets. However, the survival of larvae fed on *D. carota* and *M. chamomilla* flowers did not differ from the starvation treatment (Figure 1).

Larvae that fed on prey diets completed development in approximately 17 days (Table 2). First and second instar stages took from 2 to 3 days, third instar developmental time for larvae that fed on *M. persicae* was significantly longer than when the same larvae fed on *D. plantaginea*. The fourth instar took 4–6 days and the pupal stages lasted 4–6 days before emergence. Larvae that fed on *E. kuehniella* eggs developed significantly faster ( $\sim 1.3$  days) than those on aphid diets (Table 2).

### Adult Longevity and Fecundity

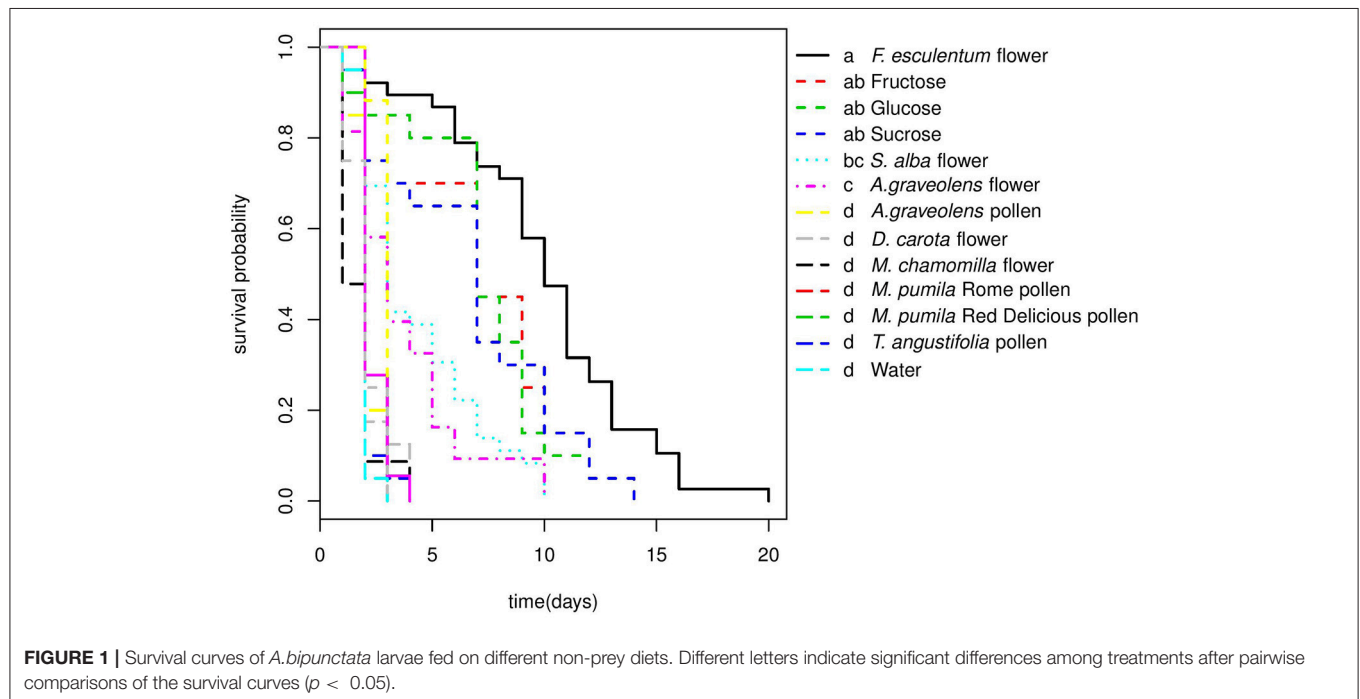
*Adalia bipunctata* adults longevity was significantly affected by diet (Cox proportional hazards,  $df = 13$ ,  $\chi^2 = 1135.9$ ,  $p < 0.0001$ ), however, no significant difference was found between females and males (Cox proportional hazards,  $df = 1$ ,  $\chi^2 = 2.1$ ,  $p = 0.15$ ) and there was no interaction between sex and diet (Table S1). Adults survived on average ( $\pm$  SE) for 4.93 ( $\pm 0.19$ ) days in the starvation treatment (water-only). Adults' longevity



**TABLE 2 |** Developmental time (days) (mean  $\pm$  SE) of *Adalia bipunctata* larvae fed on different diets.

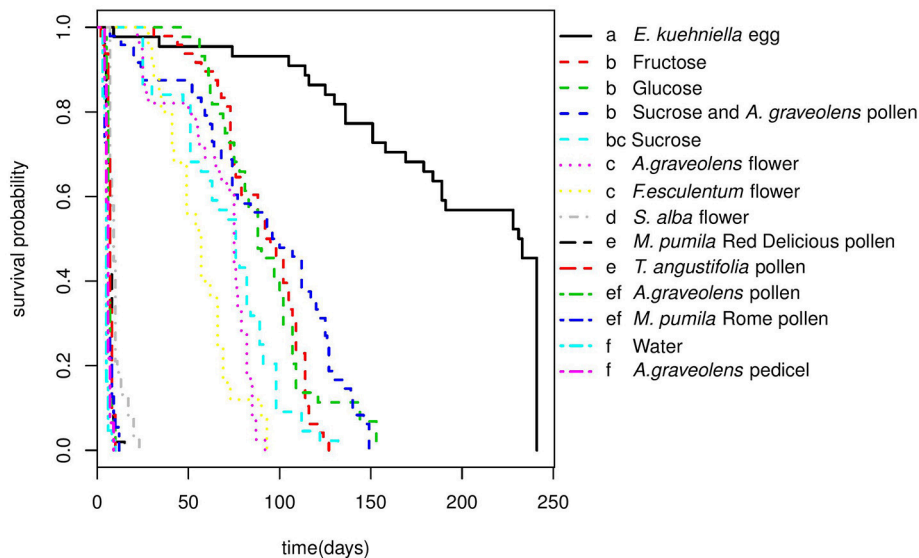
Treatments	First instar	Second instar	Third instar	Fourth instar	Pupa	Egg-adult
<b>Prey diets</b>						
<i>D. plantaginea</i>	2.33 $\pm$ 0.14 (18)ab	1.94 $\pm$ 0.06 (18)a	1.94 $\pm$ 0.10 (18)b	5.67 $\pm$ 0.16 (18)a	5.61 $\pm$ 0.12 (18)a	17.50 $\pm$ 0.26 (18)a
<i>M. persicae</i>	2.00 $\pm$ 0.16 (20)b	1.90 $\pm$ 0.22 (20)a	2.35 $\pm$ 0.13 (20)a	5.40 $\pm$ 0.23 (20)a	5.40 $\pm$ 0.11 (20)a	17.05 $\pm$ 0.09 (20)a
<i>E. kuehniella</i> eggs	2.88 $\pm$ 0.08 (17)a	1.88 $\pm$ 0.12 (17)a	2.18 $\pm$ 0.13 (17)ab	4.88 $\pm$ 0.08 (17)b	4.41 $\pm$ 0.17 (17)b	16.24 $\pm$ 0.20 (17)b
<b>Sugar solution</b>						
Fructose	7.05 $\pm$ 0.84 (20)ab	–	–	–	–	–
Glucose	7.35 $\pm$ 0.68 (20)ab	–	–	–	–	–
Sucrose	6.70 $\pm$ 0.85 (20)ab	–	–	–	–	–
<b>Flower diets</b>						
<i>A. graveolens</i>	3.65 $\pm$ 0.39 (43)c	–	–	–	–	–
<i>D. carota</i>	2.30 $\pm$ 0.11 (40)d	–	–	–	–	–
<i>F. esculentum</i>	9.97 $\pm$ 0.67 (38)a	–	–	–	–	–
<i>M. chamomilla</i>	1.65 $\pm$ 0.18 (23)e	–	–	–	–	–
<i>S. alba</i>	4.36 $\pm$ 0.43 (36)bc	–	–	–	–	–
<b>Pollen diets</b>						
<i>A. graveolens</i>	2.05 $\pm$ 0.13 (20)de	–	–	–	–	–
<i>M. pumila</i> Rome	2.10 $\pm$ 0.07 (20)de	–	–	–	–	–
<i>M. pumila</i> Red Delicious	2.00 $\pm$ 0.10 (20)de	–	–	–	–	–
<i>T. angustifolia</i>	2.10 $\pm$ 0.12 (20)de	–	–	–	–	–
Water	2.00 $\pm$ 0.07 (20)d	–	–	–	–	–

Different letters within a column indicate significant differences among survival curves ( $p < 0.05$ ). Comparisons for survival of first instar larvae were separated for prey diet and non-prey diet. The number of individuals tested in each treatment is given in parentheses ( $n$ ).



on *E. kuehniella* eggs was significantly higher than all non-prey diets (Figure 2). Among the non-prey diet treatments, highest longevity for both sexes were obtained on sugar diets followed by *A. graveolens* and *F. esculentum* flower diets. In the presence of a 1 M sugar solution, adults survived up to 3 months. For

flower diets, adults lived longer on *A. graveolens* ( $65.97 \pm 3.43$  days) and *F. esculentum* ( $56.04 \pm 3.72$  days) than on *S. alba* ( $10.11 \pm 0.66$  days) flowers. Compared to sugar and floral diets, pure pollen diets hardly improved *A. bipunctata* longevity over that of the starvation treatment. Although, for *M. pumila* Red



**FIGURE 2 |** Survival curves of *A. bipunctata* adults (females and males) fed on different diets. Different letters indicate significant differences among treatments after pairwise comparisons of the survival curves ( $p < 0.05$ ).

Delicious and for *T. angustifolia*, the difference was significant ( $z = -4.2$ ,  $p = 0.002$ ;  $z = -4.4$ ,  $p = 0.001$ ) and increased longevity from <5 days on water to around 7 days (an increase of over 40%) (Table 3). Despite adults' longevity on pure pollen diets being low, longevity on the mixed diet of *A. graveolens* pollen and sucrose solution was over 40% higher than on the pure sucrose solution diet, which was equivalent to the fructose diet and only surpassed by the *E. kuehniella* diet. There was no significant difference between females and males longevity on different diets, except for the *A. graveolens* pedicel diet, with slightly longer longevity of females ( $z = 2.3$ ,  $p = 0.02$ ; Table S2).

Only females that fed on *E. kuhniella* eggs laid eggs, with an average of  $699.05 \pm 134.00$  eggs per female, while none of the females fed on non-prey diets laid eggs (Table 3).

## Lipid Analysis

Lipid content of adults was affected by diets ( $\chi^2 = 197.5$ ,  $p < 0.0001$ ), but no significant difference was found between females and males ( $\chi^2 = 0.2$ ,  $p = 0.65$ ). The highest lipid levels were found in individuals fed on sucrose (25%), but it was not statistically different from individuals fed on *E. kuhniella* eggs or newly emerged adults for both females and males ( $p = 0.06$  and  $p = 0.26$  for females;  $p = 0.06$ , and  $p = 0.26$  for males; Table 4). The lipid content of females that fed on sucrose solution was significantly higher than females that fed on fructose or glucose. No significant difference in lipid contents was found in males. Lipid content of adults that fed on sucrose solution alone was much higher than those that fed on the mixed diets of sucrose solution and *A. graveolens* pollen ( $z = 9.7$ ,  $p < 0.0001$ ; Table 4). Adults that fed on fructose or glucose solution had the same lipid content as those that fed on *A. graveolens* flowers.

## DISCUSSION

In this study, the effect of different non-prey food sources (pollen, sugar solutions, and flowers) and prey diets on longevity and fecundity of *A. bipunctata* adults and survival of *A. bipunctata* larvae were tested. Larvae did not develop beyond the first instar in any of the non-prey diet treatments but could survive up to 10 days on a non-prey diet (Table 2). Adults fecundity was nil on all non-prey diets, but non-prey diets increased longevity up to 92 days (Table 3). High lipid content was found in adults that fed on sugar solutions (Table 4).

None of the four pollen diets differed significantly from the starvation treatment for *A. bipunctata* larval development (Figure 1). For adults, *M. pumila* (Red Delicious) and *T. angustifolia* pollen significantly increased longevity by 2 days over water, which was equivalent to a 40% increase in longevity, but still inferior to other non-prey diets (Table 3). The larval developmental rate of *A. bipunctata* is mainly dependent on the quality and quantity of food and temperature (Wratten, 1973; Omkar, 2005; Jalali et al., 2010). Hemptinne and Desprets (1986) reported that *A. bipunctata* larvae completed the development on pollen of Rosaceae, although the development time was longer than on a prey diet. Similar results were found for *Harmonia axyridis* (Pallas) provided with a frozen, moist honeybee pollen diet (Berkvens et al., 2008). *Coleomegilla maculata* (DeGeer) can develop and reproduce on a diet consisting solely of maize pollen (Lundgren and Wiedenmann, 2004). On the contrary, in a study conducted by Amala and Yadav (2013), the effects of five different diets on the developmental time of larval *Stethorus rani* (Coleoptera, Coccinellidae) were studied. They found that *S. rani* larvae that fed on a pollen diet had the lowest larval survival percentage compared to other diets and failed to develop to the pupal stage, but those fed on honey and extrafloral nectaries

**TABLE 3** | Longevity (days) and fecundity (mean  $\pm$  SE) of *A. bipunctata* adults fed on different diets.

Treatments	Longevity			Fecundity (eggs/female)
	Females	Males	Females and males	
Sugar solution				
Fructose	95.08 ± 4.25 (24)b	86.17 ± 4.95 (24)bc	90.62 ± 3.32 (48)b	–
Glucose	89.10 ± 4.58 (29)bc	98.60 ± 7.80 (15)b	92.34 ± 4.08 (44)b	–
Sucrose	69.92 ± 5.55 (25)cd	72.58 ± 6.31 (19)bcd	71.07 ± 4.17 (44)bc	–
Flower diets				
<i>A. graveolens</i>	64.17 ± 4.70 (23)d	68.56 ± 4.87 (16)cd	65.97 ± 3.43 (39)c	–
<i>F. esculentum</i>	57.29 ± 5.32 (14)d	54.45 ± 5.04 (11)d	56.04 ± 3.72 (25)c	–
<i>S. alba</i>	10.67 ± 0.88 (24)e	9.14 ± 0.89 (14)e	10.11 ± 0.66 (38)d	–
Pollen diets				
<i>A. graveolens</i>	6.60 ± 0.22 (30)f	6.23 ± 0.40 (13)fg	6.49 ± 0.20 (43)ef	–
<i>M. pumila</i> Rome	6.27 ± 0.41 (33)fg	5.27 ± 0.37 (22)fg	5.87 ± 0.29 (55)ef	–
<i>M. pumila</i> Red Delicious	7.17 ± 0.38 (30)f	6.85 ± 0.31 (20)ef	7.04 ± 0.26 (50)e	–
<i>T. angustifolia</i>	7.09 ± 0.30 (32)f	6.81 ± 0.27 (16)ef	7.00 ± 0.22 (48)e	–
Other diets				
<i>A. graveolens</i> pedicel	6.06 ± 0.23 (31)fg	5.27 ± 0.22 (15)g	5.80 ± 3.43 (39)f	–
<i>E. kuehniella</i> eggs	193.70 ± 13.44 (22)a	191.21 ± 13.42 (22)a	193.48 ± 13.29 (44)a	699.05 ± 134.00 (22)
Sucrose and <i>A. graveolens</i> pollen	100.84 ± 8.41 (25)b	83.22 ± 7.53 (23)bcd	92.40 ± 5.82 (48)b	–
Water	4.74 ± 0.19 (31)g	5.42 ± 0.45 (12)efg	4.93 ± 0.19 (43)f	–

Different letters within a column indicate significant differences among survival curves ( $p < 0.05$ , **Table S3**). Cox proportional hazard model (R function "coxph") was used. The number of individuals tested in each treatment is given in parentheses (n). No adults fed on non-prey diets lay eggs.

completed development. *Harmonia axyridis* failed to complete its development or reproduce when fed exclusively on fruit (apple, pear, and raspberries) and fungi, although larval, and adult survival were prolonged when fruit was offered compared with only water (Berkvens et al., 2010). Ladybeetles developed better when offered a mixed diet compared to pollen alone (De Clercq et al., 2005; Berkvens et al., 2008; Bonte et al., 2010; Amala and Yadav, 2013). Possible explanations for the contrasting results in this study and those of Hemptinne and Desprets (1986), may be the different nutritional value of pollen tested. Mixed pollen of Rosaceae was provided in the Hemptinne and Desprets (1986) study, but in the present study, only pollen of one plant species was provided, and the paper does not state if it was bee-pollen or hand-collected pollen. Bee-collected pollen always possesses a higher nutritional value than pollen collected by hand, because it contains larger amounts of sugars from the honey or nectar in the fluid used to cement the grains together (Lunden, 1954; Linskens and Jorde, 1997). Different nutritional requirements of the species tested could also explain inconsistencies in the results. Previous studies indicate that pollen could enable *A. bipunctata* females to promptly oviposit at the time of aphid population increase (Hemptinne and Desprets, 1986). Our results show that pollen diets increased adult longevity by 19–43% compared to starvation (water-only), but not as much as floral and sugar diets, pointing to the need for adults to have access to sugars. However, the value of pollen was also clearly shown by the fact that *A. graveolens* pollen added to a sucrose sugar diet increased adult longevity by over 30% compared to the pure sugar diet.

Sugar solutions prolonged the developmental time of larvae and improved *A. bipunctata* adult longevity (**Tables 2, 3**). Sugars are easily digestible high-energy foods, and can dramatically increase survival of coccinellids in the absence of prey (Matsuka et al., 1982; Dreyer et al., 1997). In this study, adults that fed on glucose lived longer than those that fed on sucrose. Nijima et al. (1997) mentioned that not all mono- and oligo-saccharides are equally suitable for coccinellids. Sugar-feeding did not support reproduction in this study. A similar result was reported by Smith and Krischik (1999), who found that sugar-feeding seldom supported reproduction in coccinellids on its own. However, sugar consumption can shorten pre-oviposition periods of coccinellids and help females to survive reproductive diapause (Reznik and Vaghina, 2006). The high lipid content of adults that fed on sugars (**Table 4**) supports this finding and also points to the value of sugars in building lipid reserves for survival in periods without prey and for overwintering.

*Adalia bipunctata* larval survival and adult longevity on flower diets of different species varied greatly (**Table 3**). The accessibility and quality of the nectar and pollen are important factors that affect the relative preference of natural enemies for specific plant species (Hogg et al., 2011; van Rijn and Wäckers, 2016). The nutritional composition differs among different plant species (**Table 1**). *Adalia graveolens* and *F. esculentum* flowers increased the longevity of adults compared to the starvation treatment, but did not support egg production. The results are in accordance with Togni et al. (2016), who found that when given access to coriander (*Coriandrum sativum*) flowers increased adult longevity but not reproduction of the coccinellid



**TABLE 4 |** Lipid content (%) (Mean  $\pm$  SE) of *Adalia bipunctata* adult fed on different diets.

Treatments	Lipid content %		
	Males	Females	Males and females
<b>Sugar solution</b>			
Fructose	16.94 $\pm$ 5.59 (8)abc	14.56 $\pm$ 5.17 (8)bc	15.75 $\pm$ 3.69 (16)bc
Glucose	13.81 $\pm$ 4.36 (8)abcd	7.39 $\pm$ 2.04 (8)cd	10.6 $\pm$ 2.47 (16)cd
Sucrose	23.92 $\pm$ 6.11 (7)a	25.97 $\pm$ 4.87 (8)a	25.01 $\pm$ 3.72 (15)a
<b>Flower diets</b>			
<i>A. graveolens</i>	10.17 $\pm$ 2.44 (8)bcde	7.9 $\pm$ 0.98 (8)cd	9.04 $\pm$ 1.3 (16)cde
<i>F. esculentum</i>	1.31 $\pm$ 0.28 (6)de	5.88 $\pm$ 3.68 (8)cd	3.92 $\pm$ 2.14 (14)de
<i>S. alba</i>	2.99 $\pm$ 2.06 (8)de	1.71 $\pm$ 0.5 (7)d	2.39 $\pm$ 1.1 (15)de
<b>Pollen diets</b>			
<i>A. graveolens</i>	2.14 $\pm$ 0.43 (8)de	1.93 $\pm$ 0.47 (8)d	2.04 $\pm$ 0.31 (16)e
<i>M. pumila</i> Rome	3.89 $\pm$ 2.8 (7)cde	4.17 $\pm$ 2.43 (8)cd	4.04 $\pm$ 1.78 (15)de
<i>M. pumila</i> Red	2.23 $\pm$ 0.31 (8)de	1.8 $\pm$ 0.17 (8)d	2.01 $\pm$ 0.18 (16)e
Delicious			
<i>T. angustifolia</i>	4.28 $\pm$ 1.74 (8)cde	3.8 $\pm$ 1.56 (8)cd	4.04 $\pm$ 1.13 (16)de
<b>Other diets</b>			
<i>A. graveolens</i> pedicel	1.86 $\pm$ 0.46 (8)de	2.51 $\pm$ 0.32 (7)d	2.16 $\pm$ 0.29 (15)de
<i>E. kuehniella</i> eggs	19.67 $\pm$ 1.52 (8)ab	20.85 $\pm$ 0.6 (8)ab	20.26 $\pm$ 0.8 (16)ab
Sucrose and <i>A. graveolens</i> pollen	0.72 $\pm$ 0.05 (8)e	0.66 $\pm$ 0.09 (8)d	0.69 $\pm$ 0.05 (16)e
Newly emerged adults (larvae were fed on <i>E. kuhniella</i> eggs)	21.54 $\pm$ 1.28 (6)ab	22.43 $\pm$ 1.84 (6)ab	21.99 $\pm$ 1.07 (12)ab
Water	3.49 $\pm$ 0.39 (8)de	2.6 $\pm$ 0.58 (8)d	3.05 $\pm$ 0.36 (16)de

Different letters within a column indicate significant differences among treatments ( $p < 0.05$ ). The number of individuals tested in each treatment is given in parentheses (n). General Linear Model (GLM) was used.

*Cycloneda sanguinea*. The two best-performing flowers in our study, *A. graveolens* and *F. esculentum*, both have well-exposed nectaries, making predator access easy. Sugar content is dominated by fructose and glucose in both *A. graveolens* and *F. esculentum* nectar, and *A. graveolens* has more total sugars than *F. esculentum* (Irvin et al., 2007). While we found no difference between the three sugars for larval survival, adults survived longer on fructose and glucose than on sucrose. These findings suggest that planting flowers such as *A. graveolens* and *F. esculentum* with accessible nectaries and with more fructose and glucose in the nectar would better support *A. bipunctata* than sucrose-dominated flowers.

Some compounds in nectar can be toxic or repellent to flower visitors (Adler, 2000; Wäckers, 2001). For example, glucosinolates (GLS) are present in all parts of Brassicaceae crop species (Merritt, 1996), even in nectar (Bruinsma et al., 2014), inducing deleterious effects on *A. bipunctata* (Francis et al., 2001). This may explain why *A. bipunctata* adults that fed on white mustard flower lived much shorter than those that fed on *A. graveolens* and *F. esculentum*.

Larvae performed poorly on all flower diets except *F. esculentum* (Table 2). Although considerable amounts of pollen can be found on the chamomile flower, the nectaries are hidden in a capitulum (Patt et al., 1997), so chamomile may have served as a pure pollen diet, which corresponds with the survival of larvae fed on pollen diets. Restricting the access of predators to nectar can reduce their survival and fitness considerably (Lundgren and Seagraves, 2011; Portillo et al., 2012; van Rijn and Wäckers, 2016). Feeding on *D. carota* flowers can selectively benefit some insects, such as the parasitoid *Cotesia glomerata* (Winkler et al., 2009), while some others such as the lacewing *Chrysoperla carnea* and the herbivore *Pieris rapae* (Lepidoptera: Pieridae) do not benefit (Winkler et al., 2009; Gonzalez et al., 2016). The nectar is accessible and dominated by glucose and fructose (Broussard et al., 2017) but *A. bipunctata* larvae did not benefit from *D. carota* flowers in this study. The reasons for the poor performance of *A. bipunctata* larvae provided with *M. chamomilla* and *D. carota* flower diets, as well as for the poor performance of larvae on pure pollen diets remain speculative but are consistent with the central role of sugars for *A. bipunctata* survival in the absence of prey. While pollen as a pure diet has little value for *A. bipunctata* larvae, and limited value for adults, adult longevity on a sucrose diet supplemented with pollen numerically exceeded the sum of the longevity on sucrose and pollen separately, pointing to a nutritional value of pollen in mixed diets. Evans et al. (1999) and Soares et al. (2004) also reported that ladybeetles benefited from mixed diets.

In this study, lipid content of newly-emerged adults, presumably stored during larval development, was also analyzed. The lipid content of adults that fed on sucrose solution did not differ from that of adults that fed on *E. kuhniella* eggs or newly emerged adults (larvae were fed on *E. kuhniella* eggs) and was significantly higher than of starved individuals (water only). On the contrary, the lipid content of adults fed on pollen diets was significantly lower than that of newly emerged adults but did not differ from that of starved individuals. The lipid reserves in adults fed on water or pollen diets were consumed in a few days, which suggests that sugars contribute to maintain lipid levels, but pollen does not (Table 4). In this study, lipid content of adults that fed on sucrose solution was much higher than those that fed on a mixed diet sucrose solution and *A. graveolens* pollen. On the contrary, higher lipid content has been observed in hoverflies that fed on honey and pollen, compared to those that fed on honey alone (Pinheiro et al., 2015). The inconsistency of the results could be due to the different species tested or to *A. bipunctata*'s physiological processes. This should be addressed in future studies.

The results of the present study emphasize the importance of non-prey foods for *A. bipunctata* in agroecosystems such as orchards when prey populations are low. Sugar solutions and flowering plants, especially *F. esculentum* and *A. graveolens*, can prolong *A. bipunctata* longevity, but floral diets did not support adult fecundity. Flowers and sugars could also increase immature survival, though they did not support molting. Whether flowering plants attract or retain *A. bipunctata* in a habitat *per se* is not known, but our results show that longevity of *A. bipunctata* is greatly prolonged by floral diets. In the field,

flowering plants may also host alternative prey, which would support both larval development and adult fecundity. Flowering plants can enhance the effectiveness of natural enemies by increasing natural enemies' longevity, fecundity, and predation or parasitism rates (Lee and Heimpel, 2008; Russell, 2015; van Rijn and Wäckers, 2016), and have been widely used in conservation biological control (Fiedler et al., 2008; Haaland et al., 2011). Addison et al. (2000) found pollen to be abundant on apple leaves very soon after the leaves had opened, and predatory mites can use windborne pollen released from cover crops in the field (Warburg et al., 2018). Nectar is also available in apple flowers during flowering in orchards (Toth et al., 2003). However, the effects of floral diets on *A. bipunctata* may decrease in the field due to various abiotic and biotic conditions (Brody, 1997; Adler et al., 2006). Analyses of lipid contents of adults that fed on different diets point to the potential of sugars to contribute toward better winter survival of the adults.

In conclusion, floral diets can prolong *A. bipunctata* longevity, which may expand biological control services by *A. bipunctata*. Further studies on whether prey deprivation might affect the future ability of *A. bipunctata* to provide biocontrol services are needed for conservation biological control. A limitation of this study is that larvae were deprived of prey at the very first instar; however, ladybeetles would lay eggs near an aphid colony and it is rare that the first instar would face the total absence of prey. Further studies on a diet switch (from prey diet to pollen diet on the third/fourth instar) would be especially relevant, since an aphid colony may become extinct before the larvae complete their development (Dixon, 1985). Further studies on prey and non-prey mixed diets are needed in order to obtain a better understanding of the value of non-prey diets in cases where prey is limited in the field. Furthermore, because of various abiotic and biotic conditions, studies on how to provide non-prey foods in cropping systems to improve biological control agents' efficiency are needed for conservation biological control.

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## DATA ACCESSIBILITY STATEMENT

The dataset supporting this article has been deposited in zenodo at: <https://doi.org/10.5281/zenodo.2546385>.

## AUTHOR CONTRIBUTIONS

XH and LS conceived the study. XH carried out the lab work and wrote the first draft of the manuscript. XH and LS interpreted the results and wrote the manuscript.

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## SUPPLEMENTARY MATERIAL

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